recurrence, but also the opportunity to identify novel targets in the dominant clone at recurrence, some of which may be eminently and imminently targetable using existing therapeutics. Considering the lethality of glioblastoma, we believe the time has come for routine biopsy at relapse in settings where targeted agents will be employed, with the goal of identifying targets still present at recurrence, and eventually to target pathways enriched at relapse upfront as anticipatory therapy.

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Keeping It in the Family: ATRX Loss Promotes Persistent Sister Telomere Cohesion in ALT Cancer Cells

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In this issue of *Cancer Cell*, Ramamoorthy and Smith report that cancer cells that maintain their chromosome ends through alternative lengthening of telomeres (ALT) display persistent sister telomere cohesion. This delayed resolution of sister telomere cohesion depends upon the loss of ATRX and its histone-sequestering function and is associated with increased recombination between sister telomeres.

Telomeres in many human somatic cells shorten with each round of replication, whereas telomeres are maintained with cell division in cancer cells. In 90% of human tumors, the enzyme telomerase is responsible for telomere maintenance and cellular immortalization. However, the remaining 10% of cancers lack telomerase expression and, in these cells, the alternative lengthening of telomeres (ALT) pathway counteracts normal shortening. ALT cells use a recombination-based mechanism to increase the length of telomeric DNA, but, for recombination to occur, telomeres must encounter each other in the nucleoplasm. One means for connecting two chromosome ends in the space of the nucleus involves one telomere traveling directionally across large distances to find another, using the machinery that normally drives meiotic chromosome synapsis (Cho et al., 2014). In this model, telomeres can rapidly travel up to 5 μm to cluster together and recombine. Alternatively, telomeres can exploit the fact that, after telomere replication in S-phase, each chromosome end has a nearby sister telomere that can serve as a template for recombination at a distance of only 0.5 μm. Mitotic cells typically use sister chromatid recombination instead of recombination between homologs. Although ALT clearly relies on telomere-telomere recombination, the relative proportion of recombination between sisters versus recombination between homologs is unknown (Dunham et al., 2000)

In this issue of *Cancer Cell*, Ramamoorthy and Smith (2015) observe that, in ALT cell lines, sister telomere cohesions that normally dissolve after S-phase persist into mitosis, leading them to hypothesize that this persistent cohesion allows the telomeres to preferentially serve as a template for recombination. Indeed, one of the core molecular features of ALT cells

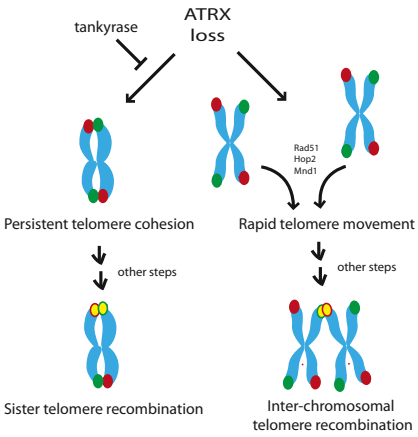

Figure 1. ATRX Loss Contributes to Both Inter-chromosomal and Sister Telomere Recombination

Loss of ATRX is linked with both increased sister telomere recombination and increased inter-chromosomal telomere recombination. In the first case, ATRX loss causes delayed resolution of sister telomere cohesion. This persistent cohesion is associated with increased recombination between sister telomeres. Both of these phenotypes can be suppressed with overexpression of tankyrase, a telomere-associated protein involved in sister telomere resolution. In the second case, telomeres recombine with a telomere on another chromosome, often moving rapidly over large inter-nuclear distances with the help of Rad51 and the meiotic proteins Hop2 and Mnd1. However, in either case, loss of ATRX is not sufficient to trigger the recombination of telomeres and other as-yet-unknown steps are required.

is an elevated rate of telomere sister chromatid exchange (T-SCE) (Londoño-Vallejo et al., 2004). This raises the possibility that ALT T-SCE is enhanced by these persistent telomere cohesions.

The SWI/SNF-like ATPase ATRX is lost in a majority of ALT-positive cell lines (Heaphy et al., 2011; Lovejoy et al., 2012). ATRX has been shown to have diverse functions, from altering patterns of methylation at repetitive genomic sequences to enhancing histone exchange. To examine the role of ATRX in persistent telomere cohesion, Ramamoorthy and Smith (2015) show that reintroduction of ATRX into ALT cell lines causes resolution of telomere cohesion and lower rates of T-SCE. Conversely, knockdown of ATRX in telomerase positive cell lines triggers the appearance of persistent sister telomere cohesion (see Figure 1). The authors make the important observation that loss of ATRX therefore phenocopies loss of tankyrase, a telomeric poly(adenosine diphosphate-ribose) polymerase (PARP), whose depletion in cancer cells leads to enhanced sister telomere cohesion and cellular arrest in anaphase of mitosis (Dynek and Smith, 2004). They show that overexpression of tankyrase restores normal resolution of sister telomere cohesion in ALT cancer cells in a similar fashion to overexpression of ATRX (see Figure 1) (Ramamoorthy and Smith, 2015).

The best characterized function of ATRX is its histone chaperone activity; ATRX and Daxx have each been shown to deposit histone H3.3 at repetitive sequences in the genome (Lewis et al., 2010). Additionally, ATRX binds to and negatively regulates deposition of the variant histone macroH2A1.1 at alpha globin sequences (Ratnakumar et al., 2012). Ramamoorthy et al. provide evidence that this histone binding domain of ATRX can repress the T-SCE phenotype and show that, in the absence of ATRX, macroH2A1.1 is bound to and negatively regulates tankyrase preventing its normal association with telomeres and its role in resolving sister telomere cohesions (Ramamoorthy and Smith, 2015).

These data lead the authors to test the consequences of forcing resolution of sister telomeres in ALT cells. They employ a system where telomeres are tagged using lacO repeats to follow inter-chromosomal telomeric recombination events. Using overexpressed tankyrase to overcome tankyrase sequestration in ALT cells, the authors find that resolution of sister telomere cohesion is accompanied by rapid inter-chromosomal exchanges indicated by proliferation of the lacO sequences to additional telomeres. Over time, however, cells with the greatest number of lacO-marked telomeres are preferentially lost, leading the authors to speculate that this rapid inter-chromosomal recombination is detrimental to cellular viability (Ramamoorthy and Smith, 2015).

The data also raise many questions. Is T-SCE the most common or preferred route of telomere recombination in an ALT cell? In which instances would a telomere travel a greater distance to recombine with a telomere on another chromosome? What are the cellular conditions and machinery that would favor one route over another? In a study by Greenberg and colleagues, induction of damage at telomeres using a Fok1 endonuclease fused to a telomeric protein caused rapid telomere movement and inter-chromosomal telomere recombination (Cho et al., 2014). Perhaps inter-chromosomal telomere recombination is favored when a telomere is damaged or when sister telomere recombination is inefficient.

Intriguingly, Karlseder and colleagues recently reported that depletion of the histone chaperone ASF-1 leads to hallmarks of ALT including promyelocytic leukemia (PML) bodies, extrachromosomal telomeric DNA, increased rates of T-SCE, and inter-telomeric recombination (O'Sullivan et al., 2014). Together with the role of ATRX/DAX in ALT, these findings solidify the idea that altered chromatin dynamics are key in the ALT mechanism. It would be interesting to examine whether the increased rates of T-SCE in ASF-1 knockdown cells are also mediated by persistent sister telomere cohesion and can be suppressed by overexpression of ATRX or tankyrase. Exploring these questions may help us understand more broadly the connection between telomeric recombination and histone chaperones.

Additionally, the exact causal role of ATRX loss in ALT cells has yet to be elucidated, and this manuscript adds another piece to the puzzle. It seems increasingly likely that altered chromatin at telomeres in ALT cells lacking ATRX contributes to rendering the telomeres more recombinogenic. The new data presented in this manuscript suggest that another possible role of ATRX loss is to promote prolonged cohesion time between sister telomeres and encourage their recombination through physical interaction. However, despite the fact that Ramamoorthy and Smith (2015) show that ATRX loss is sufficient to promote sister telomere cohesion, others have found that loss of ATRX is not sufficient alone to induce T-SCE formation or the full ALT state (Lovejoy et al., 2012). Furthermore, the nature of this cohesion induced upon ATRX knockdown remains to be explored. Is it mediated as in non-ALT cancer cells by the ring complex cohesin or does it reflect more direct association, such as strand invasion and the actual process of homologous recombination? It seems that additional events beyond ATRX loss are likely required for cells to achieve full blown ALT, and it will be important to determine specifically the steps involved.

These new findings connect a telomere-associated protein, tankyrase, with

the ATRX/Daxx pathway in ALT. It has been shown that tankyrase loss in non-ALT cells causes persistent telomere cohesions and cell arrest in anaphase of mitosis (Dynek and Smith, 2004). How do ALT cells with delayed resolution of sister telomere cohesion manage to circumvent this blockade in the cell cycle? Do other shelterin members, such as the tankyrase-binding partner TRF1, play any role in this aspect of the ALT pathway?

In ALT, the telomeres must first find each other to recombine, and they have a choice whether to shop locally or to sample further afield. What influences this choice and what the consequences of this decision are will help us to better understand the role of telomeric recombination in ALT and determine how telomerase-negative cancer cells acquire immortal growth properties.

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Path Forward for RAF Therapies: Inhibition of Monomers and Dimers

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Current BRAF inhibitors block signaling from monomeric $BRAF^{V600E}$, but not from oncogenic RAS, which requires RAF dimerization. In this issue of *Cancer Cell*, Yao and colleagues investigate why current drugs are ineffective against RAF dimers, while Peng and colleagues describe a pan-RAF inhibitor targeting both monomeric and dimeric RAF.

Mutations in RAS family members and BRAF are important cancer drivers in >30% of human malignancies, and up-regulation of canonical RAS/RAF/MEK/ERK signaling is observed in the majority of tumors. The extensive nature of oncogenic signaling through this pathway has made the identification of RAS and RAF inhibitors a top priority of drug discovery programs for over two decades. Although agents that block RAS activity remain elusive, the development of BRAF kinase inhibitors progressed steadily, with vemurafenib being the first to gain Food and Drug Administration (FDA) approval in 2011 for the treatment of malignant melanoma driven by $BRAF^{V600E}$, the most prevalent *BRAF* mutation. Vemurafenib and other first generation BRAF inhibitors exhibit good efficacy against $BRAF^{V600E}$ and have been touted as another success story for targeted therapeutics; however, several early observations tempered enthusiasm.

In particular, these drugs had little activity against tumors possessing *RAS* mutations, even though the RAF kinases are essential downstream effectors of RAS (Fedorenko et al., 2011). In cell-based assays, researchers further found that, while these inhibitors were effective at shutting down ERK signaling mediated by $BRAF^{V600E}$, they paradoxically upregulated ERK activity in the presence of oncogenic RAS (Gibney et al., 2013). Moreover, a subset of melanoma patients treated with these drugs developed secondary malignancies, many of which arise from cells harboring pre-existing *RAS* mutations. Finally, the effectiveness of current BRAF inhibitors in treating $BRAF^{V600E}$-driven melanoma is short-lived, with drug resistance invariably developing, often as a result of ERK cascade reactivation (Bucheit and Davies, 2014).

The apparent limitations to the usefulness of these drugs, however, were not without a silver lining in that they stimulated a flurry of investigation that has